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Analysis for N^2-(Pyridyloxobutyl)deoxyguanosine Adducts in DNA of Tissues Exposed to Tritium-Labeled 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-Nitrosonornicotine†

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The tobacco-specific carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (NNN) are metabolically activated to DNA binding intermediates, partially via 4-(3-pyridyl)-4-oxobutanediazohydroxide (7) or related carbonium ions. Previous studies have shown that generation of 7 from 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (11) in the presence of deoxyguanosine yields a major adduct identified as 2'-deoxy-*N*-[1-methyl-3-oxo-3-(3-pyridyl)propyl]guanosine (adduct 1). These results suggested that adduct 1 should be present in DNA of tissues that can metabolically activate NNK and NNN. In the present study, we evaluate the formation of adduct 1 and its structurally related straight-chain analogue 2'-deoxy-*N*-[4-oxo-4-(3-pyridyl)butyl]guanosine (adduct 2) in DNA of tissues of rats treated with [5-^{3}H]NNK or [5-^{3}H]NNN, and in DNA of nasal mucosa that had been cultured in medium containing [5-^{3}H]NNK or [5-^{3}H]NNN. Hepatic DNA from rats treated with [5-^{3}H]NNK was enzymatically hydrolyzed to deoxyribonucleosides and analyzed by HPLC. One of the radioactive peaks, peak E, coeluted with adduct 1. However, treatment of peak E with $NaBH_4$ resulted in the formation of products different from those produced by $NaBH_4$ treatment of adduct 1, demonstrating that adduct 1 could not be detected under these conditions. Hydrolysis of peak E with acid produced 4-hydroxy-1-(3-pyridyl)-1-butanone (9), suggesting that peak E might be adduct 2. Therefore, adduct 2 was synthesized by reaction of deoxyguanosine with 1-(3-pyridyl)butane-1,4-dione (5) in the presence of $NaCNBH_3$. Its HPLC retention time, however, was different from that of peak E. Peak E was also detected in DNA of nasal mucosa incubated with [5-^{3}H]NNK or [5-^{3}H]NNN. The HPLC chromatograms obtained upon enzymatic hydrolysis of DNA exposed to [5-^{3}H]NNK or [5-^{3}H]NNN were generally similar, indicating the operation of a common alkylation pathway via diazohydroxide 7 or a related carbonium ion. This pathway does not however lead to detectable levels of adducts 1 or 2 in DNA (detection limit using [5-^{3}H]NNK or [5-^{3}H]NNN, approximately 0.05 pmol/mg of DNA).

Introduction

NNK[1] and NNN are formed by the nitrosation of nicotine and are two of the most important carcinogens in tobacco and tobacco smoke (*1*, *2*). Low doses of NNK cause tumors in rat lung, liver, nasal mucosa, and pancreas (*3*, *4*). NNN is carcinogenic toward rat esophagus and nasal mucosa (*3*, *5*).

The initiation of the carcinogenic process is believed to result from modification of DNA. Previous studies have indicated that diazohydroxides formed by metabolic α-hydroxylation of NNK and NNN modify DNA as illustrated in Figure 1 (*6*–*10*). α-Hydroxylation of NNK at the methylene group leads to methanediazohydroxide (6), which methylates DNA. Methylated bases have been detected in liver, lung, and nasal mucosa of rats treated with NNK (*9*, *11*). α-Hydroxylation of NNK at the methyl group is expected to produce 4-(3-pyridyl)-4-oxobutanediazohydroxide (7). The same intermediate can be formed by ring opening of 2'-hydroxy-NNN (3). Metabolism studies of NNK and NNN are consistent with the formation of 7 (*6*, *7*). DNA binding studies of NNK and NNN labeled with tritium in the pyridine ring are also consistent with binding via 7 or a related carbonium ion, and possibly other electrophiles such as 8 (*10*). Hydrolysis of the DNA from these experiments yielded, among other products, keto alcohol 9, consistent with the intermediacy of 7 (*10*). However, the structures of the DNA adducts formed in these experiments are not known.

In previous studies, we have used the nitrosocarbamate 11 to investigate the reactivity of diazohydroxide 7 (*6*, *8*, *12*, *13*). The major product formed upon hydrolysis of 11 in the presence of dG was adduct 1 (*13*). It seemed likely that adduct 1 should be formed in DNA of tissues exposed to NNK and NNN. We investigated this possibility in the present study. When it became clear that adduct 1 was not detectable in DNA of tissues exposed to [5-^{3}H]NNK and [5-^{3}H]NNN, we extended our investigation to the structurally related compound adduct 2.

Experimental Section

Chemicals. [5-^{3}H]NNK, which has tritium at the 5-position of the pyridine ring (95% pure), and [5-^{3}H]NNN (95% pure) were obtained from Chemsyn Science Laboratories, Lenexa, KS.

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[1] Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosonornicotine; dG, 2'-deoxyguanosine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; HPLC, high-performance liquid chromatography; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; COSY, two-dimensional correlated spectroscopy; DCI, desorptive chemical ionization.

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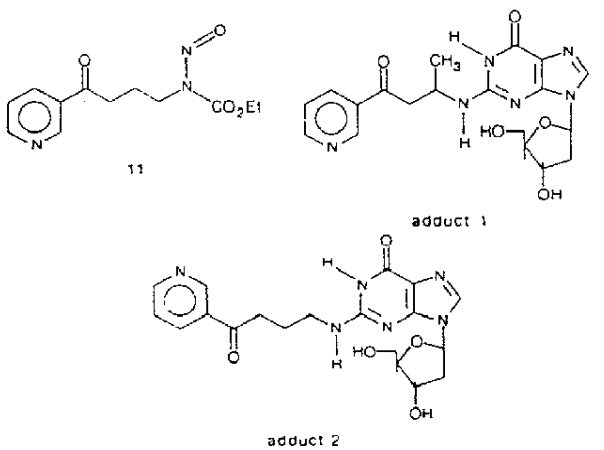

Adduct 1 was prepared by reaction of α,β-unsaturated ketone 12 with dG as described (*13*). 4-Hydroxy-1-(3-pyridyl)-1-butanone (9), 1-(3-pyridyl)-1,4-butanediol, and 1-(3-pyridyl)butan-1,4-dione (5) were synthesized (*7*, *14*, *15*). Adduct 2 was synthesized as follows: dG (400 mg, 1.5 mmol), 1-(3-pyridyl)butane-1,4-dione (250 mg, 1.5 mmol), sodium cyanoborohydride (63 mg, 1 mmol), and anhydrous $MgSO_4$ (120 mg, 1 mmol) were stirred in 20 mL of anhydrous DMF under a nitrogen atmosphere at room temperature for 2 weeks. The reaction was followed by reverse-phase HPLC, under conditions described below, except that the gradient was run in 50 min. The retention time of dG was 20 min, 1-(3-pyridyl)butan-1,4-dione, 31 min, and adduct 2, 41 min. Methylene chloride was added, and the resulting precipitate was filtered and redissolved in MeOH. The MeOH was evaporated, and the product was purified by reverse-phase HPLC on a Whatman 10 ODS-3 column with elution by 30% MeOH/H_2O at 3 mL/min: dG eluted at 8 min and adduct 2 at 25 min. This procedure yielded 20 mg of adduct 2 (3% yield): ^1H NMR (360 MHz, CD_3SOCD_3) δ 9.10 (1 H, d, J = 1.9 Hz, 2-pyridyl), 8.76 (1 H, d/d, J = 4.8, 1.8 Hz, 6-pyridyl), 8.34 (1 H, d/t, J = 7.8, 1.9 Hz, 4-pyridyl), 7.81 (1 H, s, 8-guanine), 7.55 (1 H, d/d, J = 8.0, 4.6 Hz, 5-pyridyl), 6.14 (1 H, d/d, J = 8.2, 6.4 Hz, 1′-H), 5.28 (1 H, broad, 3′-OH), 4.96 (1 H, broad, 5′-OH), 4.36 (1 H, m, 3′-H), 3.81 (1 H, m, 4′-H), 3.58 (1 H, m, 5′-H), 3.50 (1 H, m, 5′-H), 3.18 (2 H, t, J = 7.0, $CH_2C{=}O$), 2.66 (2 H, m, $CH_2CH_2CH_2$), 2.17 (1 H, m, 2′-H), 1.91 (1 H, m, 2′-H).

The DCI mass spectrum of adduct 2 was obtained on a Hewlett-Packard Model 5988 mass spectrometer (Corvallis, OR) operated in the chemical ionization mode with isobutane (0.3 Torr) as the reagent gas. Adduct 2 was dissolved in 10 μL of 2 M ammonium formate and applied to the Pt filament of the DCI probe (Vacumetrics, Inc., CA, catalog no. 31640). After the drop had evaporated, the probe was inserted into the source, the scan was begun, and the current through the Pt filament was instantaneously ramped to 2.5 A. Mass spectrum, m/e (relative intensity) 415, M + 1 (1.9), 397 (2.8), 374 (0.8), 366 (1.1), 335 (2.0), 313 (2.1), 299 (3.3), 250 (16), 210 (42), 168 (79), 166 (100).

All other chemicals and enzymes were obtained from either Aldrich Chemical Co., Milwaukee, WI, or Sigma Chemical Co., St. Louis, MO.

Animal Treatments. Male F344 rats weighing 250–300 g were obtained from Charles River Breeding Laboratories, Kingston, NY. Two rats were each given a daily ip injection of [5-^{3}H]NNK (1.72 μmol, 1.67 mCi) in 0.6 mL of saline, for 3 days. Twenty-four hours after the final injection, the animals were sacrificed and liver, lung, nasal mucosa, kidneys, and esophagus were excised for DNA isolation. The experiment was repeated four times. For examination of NNN–DNA adducts, two rats were each given a daily ip injection of [5-^{3}H]NNN (0.407 μmol, 1.11 mCi) in 0.2 mL of saline, for 3 days. The animals were sacrificed 24 h after the last injection, and liver, lung, nasal mucosa, esophagus, and kidneys were excised. The experiment was repeated two times.

Incubations of [5-^{3}H]NNK or [5-^{3}H]NNN with Cultured Nasal Mucosa. Nasal septa with their covering mucosa were excised from male F344 rats and cultured as described (*16*). Twelve septa were cultured, three per dish. Each dish contained 5 mL of medium to which was added [5-^{3}H]NNK (1.42 μmol, 1.34 mCi) in 0.2 mL of saline. Incubations were carried out for 24 h, after which DNA was isolated from the combined mucosa.

For the experiments with [5-^{3}H]NNN, each of six dishes contained three septa in 5 mL of medium to which 0.305 μmol, 0.83 mCi, of [5-^{3}H]NNN had been added. The incubations were carried out for 21 h, and the DNA was isolated. The experiment was repeated three times.

DNA Isolation and Analysis. DNA was isolated by the modified Marmur method, as described (*9*, *10*). In some experiments the DNA was further purified by anion-exchange HPLC on a Waters Associates Protein Pak 5PW column.[2] Peak E was present in enzyme hydrolysates of DNA purified by either method, and keto alcohol 9 was present in the corresponding acid hydrolysates. DNA was hydrolyzed enzymatically to deoxyribonucleosides by sequential treatment at 37 °C with DNase I (2.5 mg/mg of DNA), 4 h; alkaline phosphatase (2 units/mg of DNA), 16 h; phosphodiesterase II (0.5 units/mg of DNA), 1 h; phosphodiesterase I (0.05 units/mg of DNA), 1 h; and alkaline phosphatase (0.4 units/mg of DNA), 2 h, in 10 mM Tris, 5 mM $MgCl_2$, pH 7.0. In one of the in vivo experiments with [5-^{3}H]NNK, a different set of hydrolysis conditions was used, as follows. DNA (18 mg) was dissolved in 6 mL of 10 mM MOPS and 5 mM $MgCl_2$, pH 7.0, containing 1 mg of DNase I (2100 units), 1.2 mg of DNAse II (1080 units), phosphodiesterase I (0.8 unit), phosphodiesterase II (1.0 unit), and alkaline phosphatase (100 units). The mixture was incubated at 22 °C for 5 h. The results obtained with this method of hydrolysis were the same as those obtained with the method described above.

Hydrolysates were analyzed by HPLC using two 3.9 mm × 30 cm μBondapak C_{18} columns (Millipore, Waters Division, Milford, MA) connected in series and eluted with a 100-min linear gradient from 0 to 50% MeOH in 0.02 M pH 7.0 phosphate buffer, at 1 mL/min. Adducts 1 and 2 were detected by UV at 254 nm. Normal-phase analyses of keto alcohol 9 were carried out as previously described (*10*). Radioactivity was detected either by scintillation counting of 1-mL fractions or with a radioactive flow detector (Radiomatic Instruments, Tampa, FL).

Results

Rats were treated with [5-^{3}H]NNK, and 24 h after the final injection, they were sacrificed and DNA was isolated. The DNA was hydrolyzed enzymatically to deoxyribonucleosides, and the hydrolysates were analyzed by HPLC. Figure 2 illustrates a chromatogram obtained from hepatic DNA. The radioactivity eluting from HPLC accounted for only 47% of the radioactivity in the DNA. The remainder of the radioactivity apparently became bound to the enzymes used in the DNA hydrolysis. In some samples, the keto alcohol 9 (up to 1.4 pmol/mg of DNA), which eluted at 56 min under the conditions shown in Figure 2, was also detected. In the present study, we focused on peak E because it coeluted with adduct 1, the major product formed by reaction of nitrosocarbamate 11 with dG. Peak E was detected in hepatic DNA (0.2 ± 0.07 pmol/mg of DNA) and lung DNA (0.4 pmol/mg of DNA) of rats treated with [5-^{3}H]NNK. No peaks were detected upon HPLC analysis of enzymatically hydrolyzed DNA from nasal mucosa, kidney, or esophagus of these rats.

Hydrolysis of the hepatic DNA with 0.8 N HCl at 80 °C for 6 h gave a chromatogram similar to that previously described, in which the major peak was keto alcohol 9 (*10*). In contrast to the above results obtained by enzymatic hydrolysis, HPLC analysis of the acid hydrolysate accounted for all the radioactivity present in the DNA.

Further studies were carried out to determine whether peak E and adduct 1 were identical. Adduct 1 is a mixture of diastereomers which are only slightly separated under the HPLC conditions used in Figure 2 (*13*). Treatment

[2] S. E. Murphy, *Carcinogenesis*, in press.

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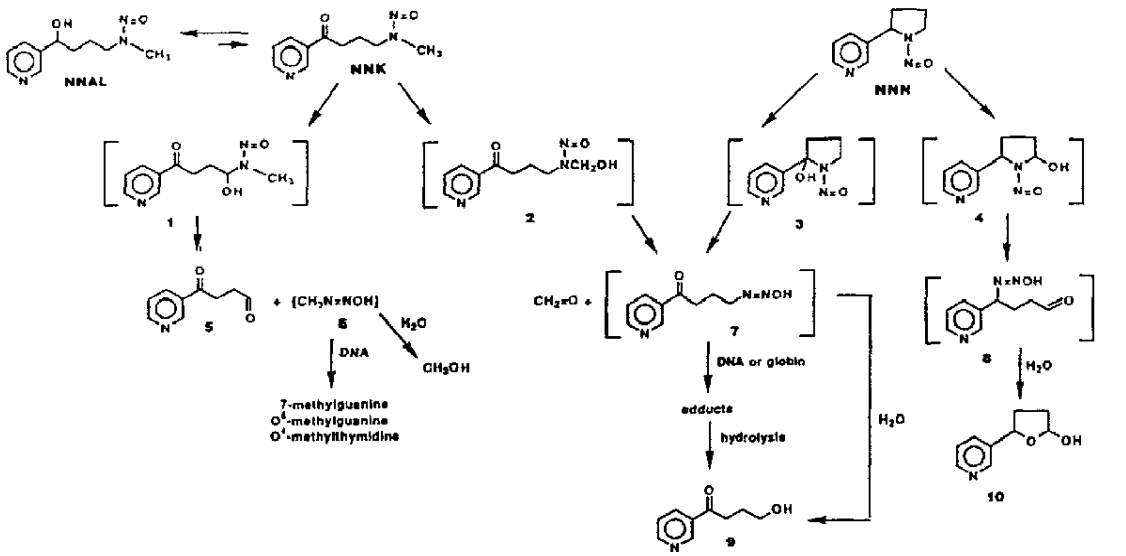

Figure 1. Enzymatic activation of NNK and NNN to metabolites and to hypothetical intermediates (shown in brackets) that bind to DNA.

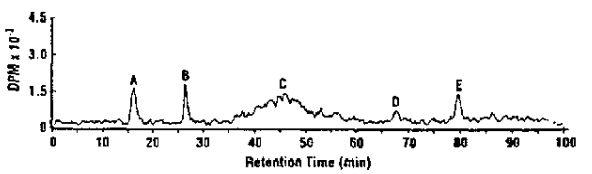

Figure 2. Chromatogram obtained upon HPLC analysis of an enzymatic hydrolysate of DNA isolated from the liver of a rat treated with [5-^{3}H]NNK.

of adduct 1 with $NaBH_4$ resulted in a chromatogram containing the four peaks 1–4 as illustrated in Figure 3. The four peaks resulted from the introduction of a new center of asymmetry by reduction of the carbonyl group of adduct 1 (*13*). Peak E of Figure 2, isolated from the hepatic DNA of the rats treated with [5-^{3}H]NNK, was mixed with standard adduct 1 and treated with $NaBH_4$. This gave the radiochromatogram illustrated in Figure 3. The radioactivity did not coelute with any of the $NaBH_4$-treated adduct 1 peaks. These results demonstrate that peak E of Figure 2 is not adduct 1.

Peak E was then treated with 0.8 N HCl at 80 °C for 6 h. A new peak was produced, which coeluted with the keto alcohol 9 under reverse-phase HPLC conditions. The identity of this peak as 9 was confirmed by cochromatography with standard 9 under normal-phase HPLC conditions. Keto alcohol 9 was not produced upon base treatment of peak E. Since the retention time of peak E was identical with that of adduct 1, and acid hydrolysis of this peak gave keto alcohol 9, we speculated that peak E might be the straight-chain analogue of adduct 1, namely, adduct 2. Therefore, adduct 2 was synthesized by reaction of 1-(3-pyridyl)butane-1,4-dione (5) with dG, followed by reduction with $NaCNBH_3$.

The spectral properties of adduct 2 were in accord with its structure. The resonances in the ^{1}H NMR spectrum of adduct 2 were assigned by comparison of the spectra of dG and 9 with that of adduct 2 and examination of the proton–proton COSY spectrum of adduct 2. All the protons were assigned unambiguously except for those on the butyl chain. Only two of the three butyl chain resonances were observed in the ^{1}H NMR spectrum (at 3.18 and 2.66 ppm). The other resonance, however, was shown to overlap

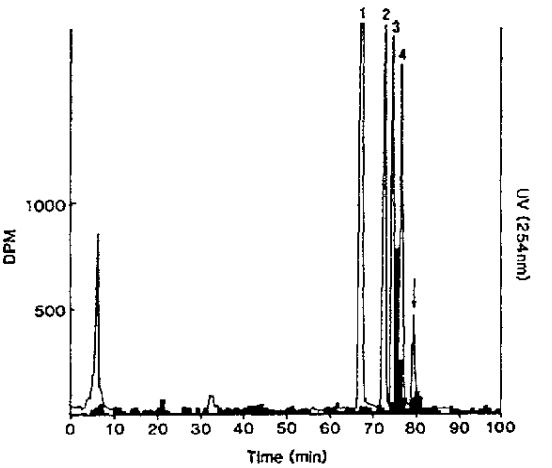

Figure 3. Chromatogram obtained upon HPLC analysis of a mixture of adduct 1 and peak E of Figure 2 that had been allowed to react with $NaBH_4$. Peaks 1–4 were detected by UV at 254 nm and represented the four diastereomers of adduct 1 in which the carbonyl group has been reduced. The histogram is radioactivity resulting from $NaBH_4$ reduction of peak E of Figure 2. The arrow represents the retention time of peak E prior to reduction, as marked by adduct 1 added to the mixture immediately prior to HPLC analysis.

with the H_2O peak at 3.3 ppm by the proton–proton COSY spectrum. The peaks at 3.18 and 2.66 ppm were assigned to the protons at the 3-position and 2-position of the butyl chain, respectively, by comparison with the spectrum of 9. The resonance under the H_2O peak was therefore assigned to the protons at the 1-position. The DCI mass spectrum of adduct 2 exhibited the M + 1 peak at m/e 415.

Adduct 2 eluted 30 s earlier than adduct 1 and peak E under the conditions used in Figure 2, demonstrating that the radioactive material formed in vivo from [5-^{3}H]NNK was not adduct 2.

The formation of DNA adducts from [5-^{3}H]NNK was further investigated by incubating it with cultures of rat nasal mucosa, under conditions similar to those described

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diazohydroxide 7, as illustrated in Figure 1. Previous studies have shown that the keto alcohol 9 as well as the corresponding acid are major metabolites of NNN and NNK in cultured nasal mucosa, which is consistent with DNA binding by this pathway. Although we have repeatedly observed keto alcohol 9 and peak E in enzymatic hydrolysates of DNA isolated from nasal mucosa cultured with [5-^{3}H]NNK or [5-^{3}H]NNN in vitro, we have not detected these products in DNA isolated from nasal mucosa of rats treated with these nitrosamines, upon either enzymatic or acid hydrolysis. It is nevertheless likely that this pathway occurs in the nasal mucosa in vivo, but to an extent too small to detect by the methods employed to date.

In summary, this study has shown that a common unknown, peak E, is detected in enzymatic hydrolysates of DNA isolated from tissues exposed to [5-^{3}H]NNK or [5-^{3}H]NNN. Peak E is not identical with adduct 1, the major product formed by hydrolysis of the model compound, nitrosocarbamate 11, in the presence of dG, nor is it identical with adduct 2, the straight-chain analogue of adduct 1. The formation of keto alcohol 9 upon acid hydrolysis of peak E indicates that it is produced from NNK and NNN through the common intermediacy of diazohydroxide 7 or a related carbonium ion.

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Registry No. Adduct 1, 120851-19-6; adduct 2, 120789-94-8; NNK, 64091-91-4; NNN, 16543-55-8; dG, 961-07-9; 1-(3-pyridyl)butane-1,4-dione, 76014-80-7.

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previously, in which extensive metabolism of NNK or NNN occurs (*16*). The DNA was isolated, enzymatically hydrolyzed, and analyzed by HPLC as in the experiments described above. Radioactive peaks eluted at 62, 71, 81, 94, and 103 min. The peak eluting at 62 min (0.6 pmol/mg of DNA) was identified as the keto alcohol 9 by cochromatography with a standard on both reverse-phase and normal-phase HPLC. The peak eluting at 81 min (0.8 pmol/mg of DNA) coeluted with adduct 1. $NaBH_4$ reduction of this material, in admixture with authentic adduct 1, produced an HPLC chromatogram essentially identical with that illustrated in Figure 3, indicating that this in vitro adduct was identical with Peak E formed in vivo from [5-^{3}H]NNK.

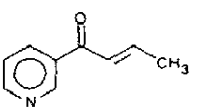
In rats treated with [5-^{3}H]NNN, DNA binding in various tissues was observed as follows (pmol/mg of DNA): liver (0.54); lung (0.50); nasal mucosa (0.02); kidney and esophagus (<0.005). HPLC analysis of enzymatic DNA hydrolysates obtained from livers of rats treated with [5-^{3}H]-NNN gave chromatograms that had two peaks, one eluting from 40 to 50 min (0.2 pmol/mg of DNA) and the other at 56 min (0.05 pmol/mg of DNA). Peak E was not detected. Only 48% of the radioactivity applied to the column eluted from the column. The peak eluting at 56 min was identified as keto alcohol 9 by coelution with a standard and by $NaBH_4$ reduction, which produced a radioactive peak coeluting with standard 1-(3-pyridyl)-1,4-butanediol.

Hydrolysates of DNA isolated from nasal mucosa cultured with [5-^{3}H]NNN contained radioactive peaks (pmol/mg of DNA) eluting at 30 (0.5), 45 (0.2), 56 (0.4), 69 (0.3), and 82 (0.2) min. Only 45% of the radioactivity applied to the column was eluted. The peak at 56 min was identified as the keto alcohol 9 by cochromatography and $NaBH_4$ reduction, as above. The peak eluting at 82 min was identical with peak E of Figure 2. Treatment of this material with $NaBH_4$ gave a chromatogram essentially identical with that illustrated in Figure 3.

Discussion

In previous studies of DNA binding in rats treated with [5-^{3}H]NNK or [5-^{3}H]NNN, we have isolated DNA from various tissues and have hydrolyzed it with either 0.8 N HCl at 80 °C or by treatment at 100 °C, pH 7.0 (*10*). Under these conditions, keto alcohol 9 was the major product identified, accounting for approximately 50% of the radioactivity present in the DNA. This is evidence for 4-(3-pyridyl)-4-oxobutylation of DNA, via diazohydroxide 7 or a related carbonium ion. However, the structure of the adduct precursor to keto alcohol 9 has not been determined. In the present study, we hydrolyzed the DNA enzymatically, or with acid. HPLC analysis of the enzymatic hydrolysates of hepatic DNA from rats treated with [5-^{3}H]NNK gave chromatograms which typically resembled that illustrated in Figure 2; the amounts of keto alcohol 9 detected under these conditions ranged from not detected, as in Figure 2, to 1.4 pmol/mg of DNA. The latter was only 25% of keto alcohol 9 released upon strong acid hydrolysis of this DNA. Similar results were obtained in the in vivo experiments with [5-^{3}H]NNN and in the nasal mucosa culture experiments. The amounts of keto alcohol 9 released by acid hydrolysis corresponded to the amount of radioactivity that was not eluted from the column upon HPLC analysis of the enzyme-hydrolyzed DNA and that apparently became bound to the hydrolyzing enzymes. These results indicate that there is a relatively unstable major adduct in DNA of tissues exposed to [5-^{3}H]NNK or [5-^{3}H]NNN which is not detected by the enzymatic hydrolysis conditions and HPLC analysis used in the present study, but which is hydrolyzed to keto alcohol 9 under strong acid or neutral thermal conditions. The structure of this adduct is under investigation. In contrast, a radioactive peak eluting at about 80 min, peak E, was detected in enzymatic hydrolysates, but not in strong acid or neutral thermal hydrolysates of DNA isolated from tissues of rats treated with [5-^{3}H]NNK or nasal mucosa cultured with either [5-^{3}H]NNK or [5-^{3}H]NNN. The absence of peak E in the strong acid hydrolysates is due to its conversion to keto alcohol 9 upon acid hydrolysis, as described under Results. Since peak E coeluted with adduct 1, we initially thought that they might be identical and consequently focused our efforts on this hypothesis.

Adduct 1 is the major product formed when the nitrosocarbamate 11 is hydrolyzed in the presence of dG. This hydrolysis is expected to proceed through the diazohydroxide 7. One of the products of this reaction was the α,β-unsaturated ketone 12, which also produces adduct 1 upon reaction with dG. Thus, adduct 1 formed in the in vitro reaction of 11 with dG may be formed mainly via 12.



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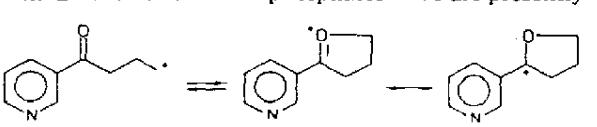
Despite the coincidence in retention times of adduct 1 and peak E, the $NaBH_4$ reduction experiments clearly demonstrated that peak E was comprised mainly of material that was not adduct 1. Thus, we did not detect adduct 1 in DNA isolated from rats treated with [5-^{3}H]-NNK or [5-^{3}H]NNN, or in DNA from nasal mucosa cultured with these nitrosamines. The detection limit was approximately 0.05 pmol of adduct 1/mg of DNA. The low yields of adduct 1 in these experiments could result from scavenging of the α,β-unsaturated ketone 12 by cellular thiols. In other experiments, we have observed that 12 reacts rapidly with *N*-acetylcysteine. We are presently using ^{32}P postlabeling to further examine the possible presence of adduct 1 in DNA of rats treated with NNK or NNN.

Acid-catalyzed hydrolysis of peak E gave the keto alcohol 9. This suggested that a 4-(3-pyridyl)-4-oxobutyl group was attached to a nucleoside or base residue. Comparison to adduct 2 demonstrated that the point of attachment of this group was not N^2 of dG. Considering the relatively small amount of peak E that is formed from NNK or NNN, the only practical approach toward its identification is the synthesis of various markers which could result from interaction of either diazohydroxide 7 or its decomposition products, carbonium ions 13 or 14, with DNA nucleosides or phosphates. We are presently



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carrying out experiments to assess the relative importance of 7, 13, or 14 in DNA binding, in order to guide further synthetic approaches.

In the experiments with cultured nasal mucosa, peak E was detected in the DNA isolated from cultures containing either [5-^{3}H]NNN or [5-^{3}H]NNK. These chromatograms also contained keto alcohol 9, as one of the major peaks. These results are consistent with the common pathway of NNN and NNK metabolism which proceeds through

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